

Supplementary Materials

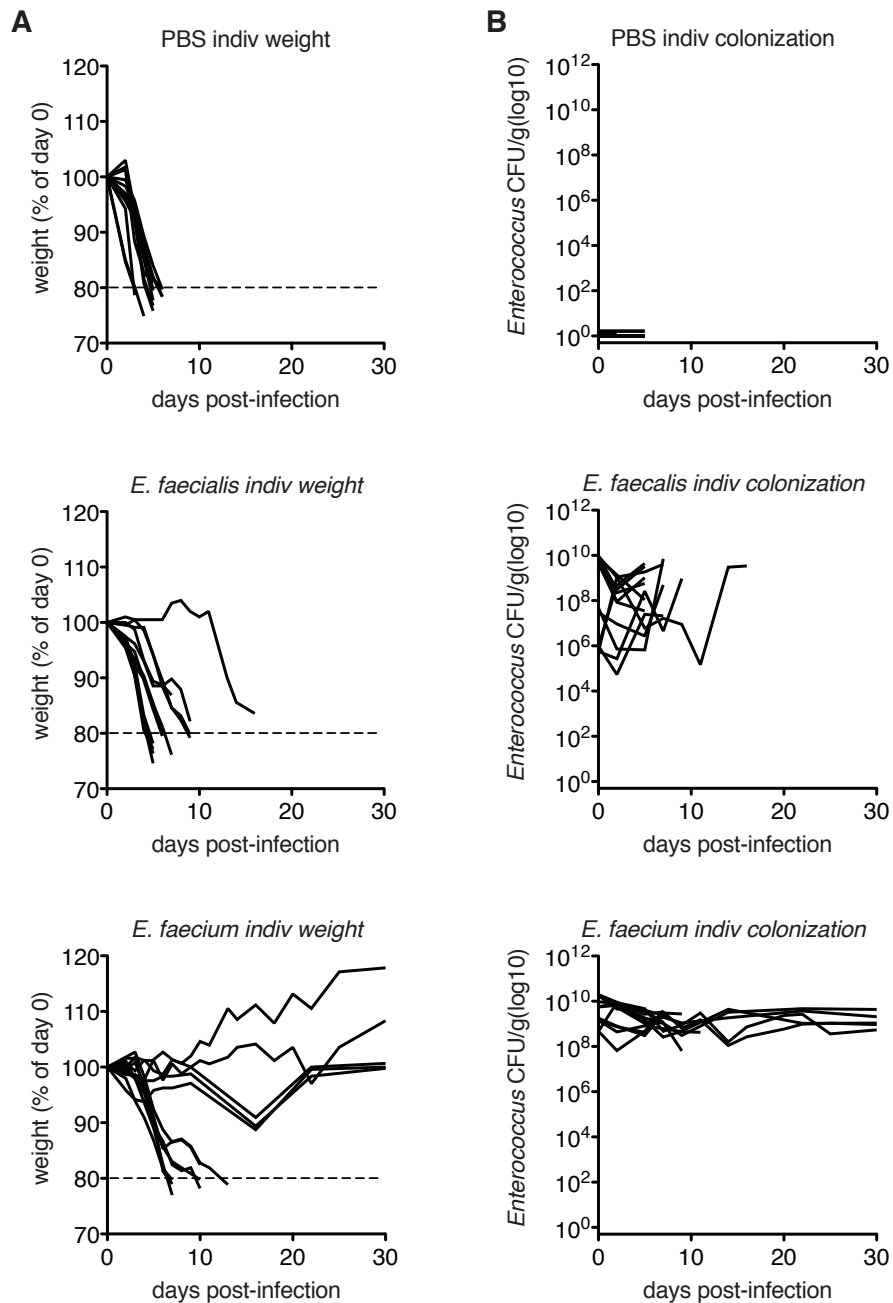


Figure S1. Individual weight loss and colonization of germ-free mice. Germ-free (GF) C57BL/6 mice were orally gavaged with 10^8 CFU *E. faecalis* or *E. faecium* 7d before oral infection with 10^2 CFU *S. Tm*. (A) Weight loss and (B) *Enterococcus* CFU present in feces shown for each mouse included in Fig. 1A-C. Pooled data from 4 independent experiments, n=12 mice/group.

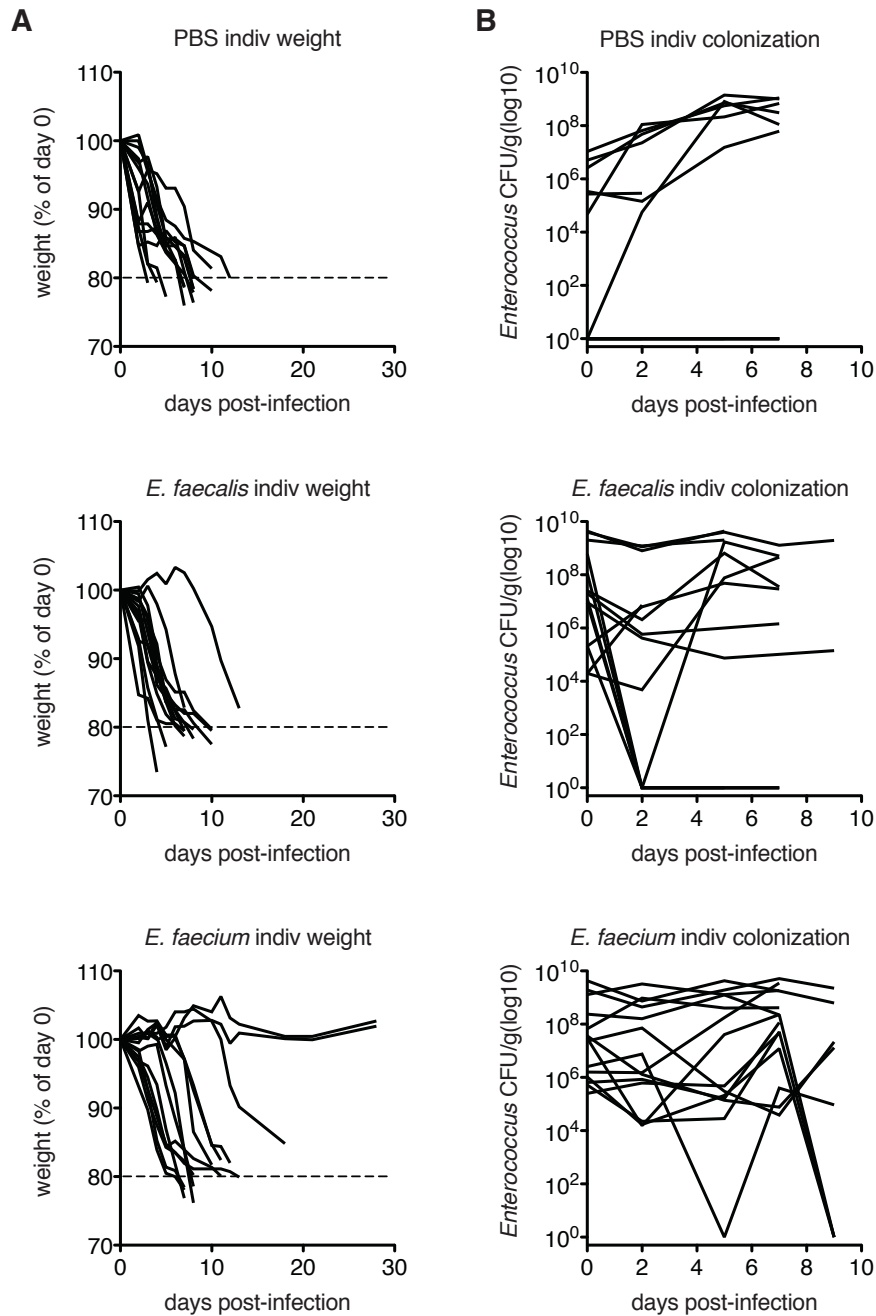


Figure S2. Individual weight loss and colonization of antibiotic-treated mice. C57BL/6 mice were orally gavaged with a broad-spectrum antibiotic cocktail of ampicillin, metronidazole, neomycin, and vancomycin (AMNV) daily for 7d prior to gavage with 10^8 CFU *E. faecalis* or *E. faecium* or PBS, followed by infection with 10^6 *S. Tm*. (A) Weight loss and (B) *Enterococcus* CFU present in feces shown for each mouse included in Fig. 1D-F. Pooled data from 4 independent experiments, n=14 mice/group.

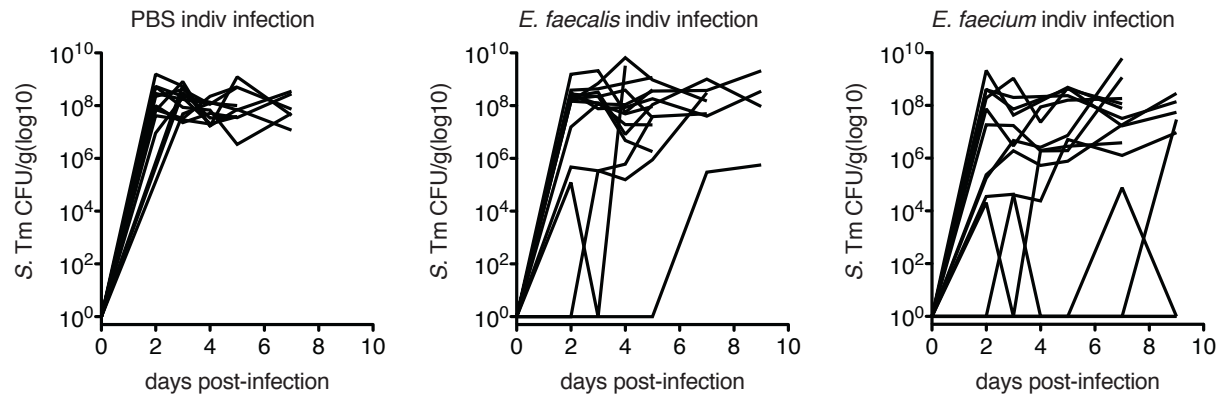


Figure S3. Individual pathogen burden in antibiotic-treated mice. C57BL/6 mice were orally gavaged with a broad-spectrum antibiotic cocktail of ampicillin, metronidazole, neomycin, and vancomycin (AMNV) daily for 7d prior to gavage with 10^8 CFU *E. faecalis* or *E. faecium* or PBS, followed by infection with 10^6 *S. Tm*. *S. Tm* bacterial burden in feces shown for each mouse included in Fig. 1D-F. Pooled data from 4 independent experiments, n=14 mice/group.

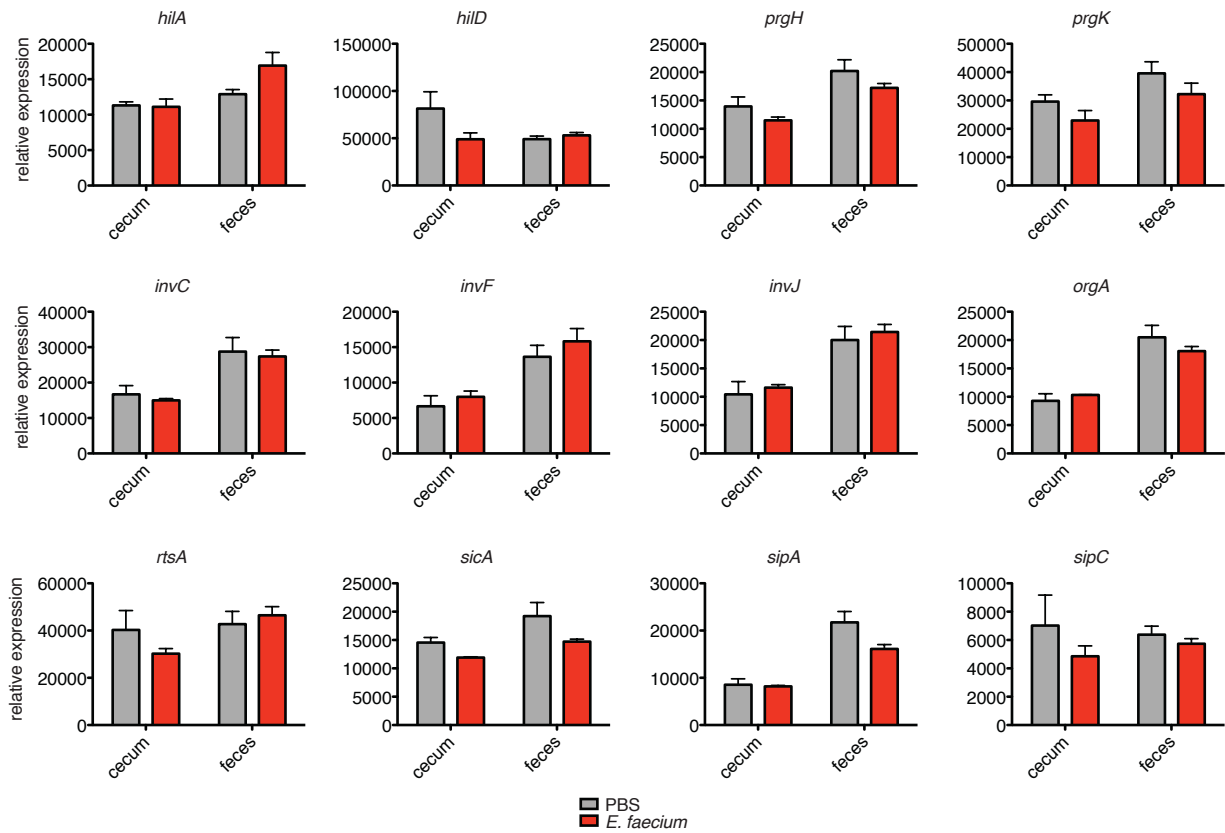


Figure S4. *E. faecium* colonization does not affect expression of *S. Typhimurium* pathogenicity genes *in vivo*. C57BL/6 mice were orally gavaged with streptomycin and given sterile PBS or 10^8 CFU *E. faecium* 4h before oral infection with 10^6 CFU *S. Tm*. Feces and cecum contents were harvested at 48h post-infection and bacterial RNA was isolated and analyzed by RQ-PCR for expression of indicated genes. Representative data, n=3 mice/group, mean \pm SEM, 2-way ANOVA with Bonferroni post-test for differences between treatments in each tissue. All treatment p-values were >0.05 and therefore not considered significant.

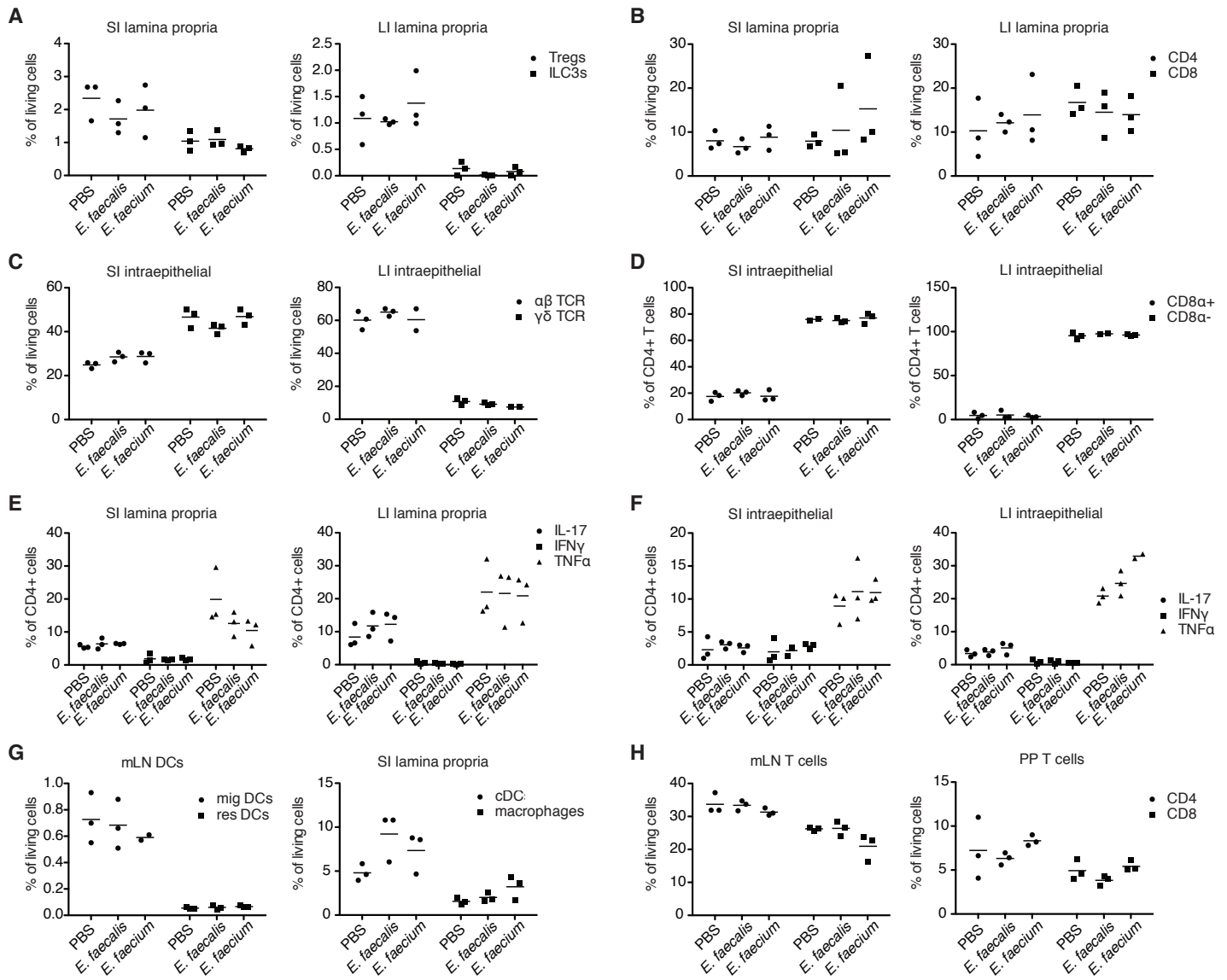


Figure S5. *E. faecium* colonization does not alter intestinal immune cell subsets. (A-H) C57BL/6 mice were gavaged with streptomycin 24h prior to gavage with PBS or 10^8 CFU *E. faecalis* or *E. faecium*. 3d post-colonization, immune cells were isolated from small intestine (SI), large intestine (LI), mesenteric lymph nodes (mLN) and Peyer's patches (PP) and analyzed by flow cytometry for relative frequency of indicated subpopulations and cytokine profile. Representative data from 1 of 3 independent experiments, n=3 mice/group. (A-H) 1-way ANOVA with Bonferroni post-test comparing all to PBS. No comparisons showed treatment to be a significant source of variation.

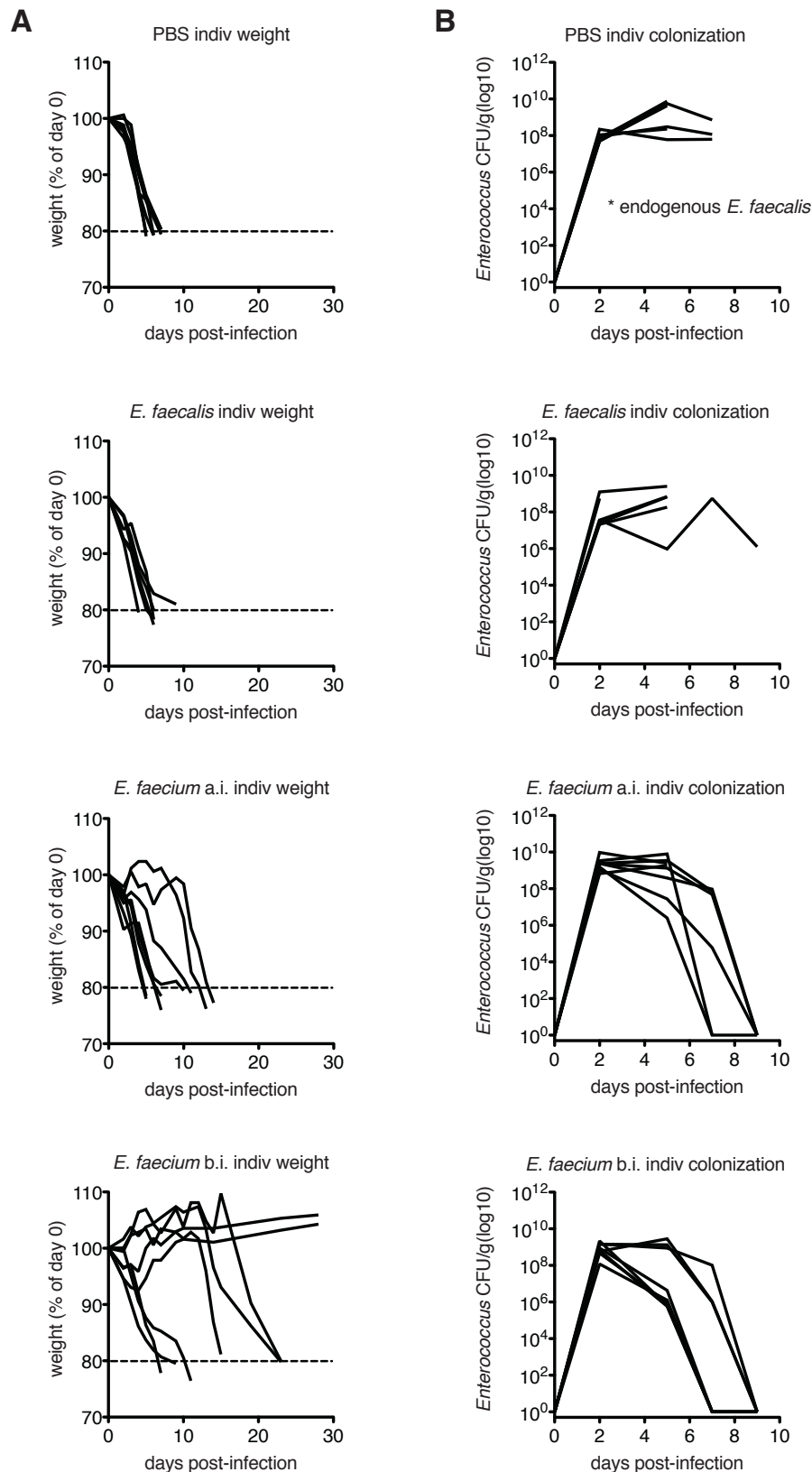


Figure S6. Individual weight loss and colonization of streptomycin-treated mice. C57BL/6 mice were orally gavaged with streptomycin and given sterile PBS or 10^8 colony-forming units (CFU) *E. faecalis* or *E. faecium* 4h before (b.i.) or 24h after (a.i.) oral infection with 10^6 CFU *S. Typhimurium* (*S. Tm*). (A) Weight loss and (B) *Enterococcus* CFU present in feces shown for each mouse included in Fig. 2A-C. Pooled data from 3 independent experiments, $n=6-8$ mice/group. For PBS controls, endogenous *E. faecalis* from returning microflora, post-antibiotic treatment, are shown.

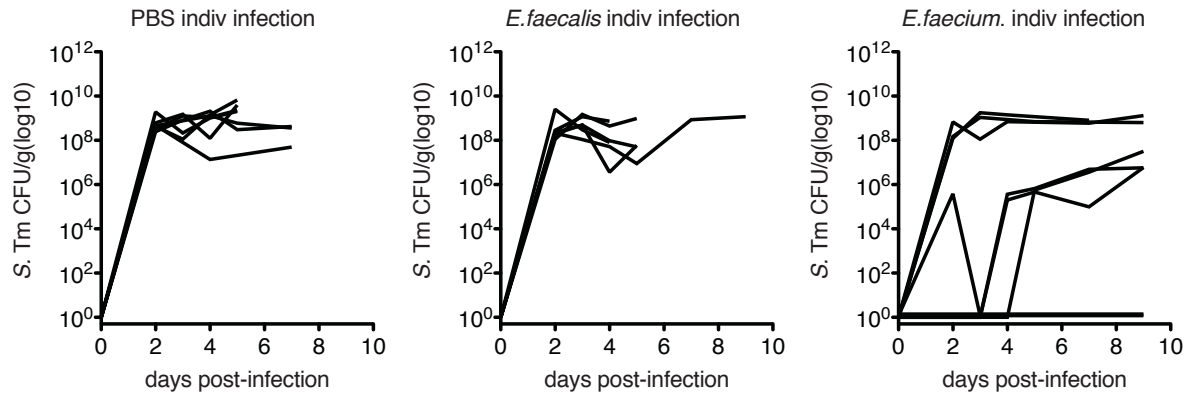


Figure S7. Individual pathogen burden in streptomycin-treated mice. C57BL/6 mice were orally gavaged with streptomycin and given sterile PBS or 10⁸ CFU *E. faecalis* or *E. faecium* 4h before oral infection with 10⁶ CFU *S. Tm*. *S. Tm* bacterial burden in feces shown for each mouse included in Fig. 2A-C. Pooled data from 3 independent experiments, n=6-8 mice/group.

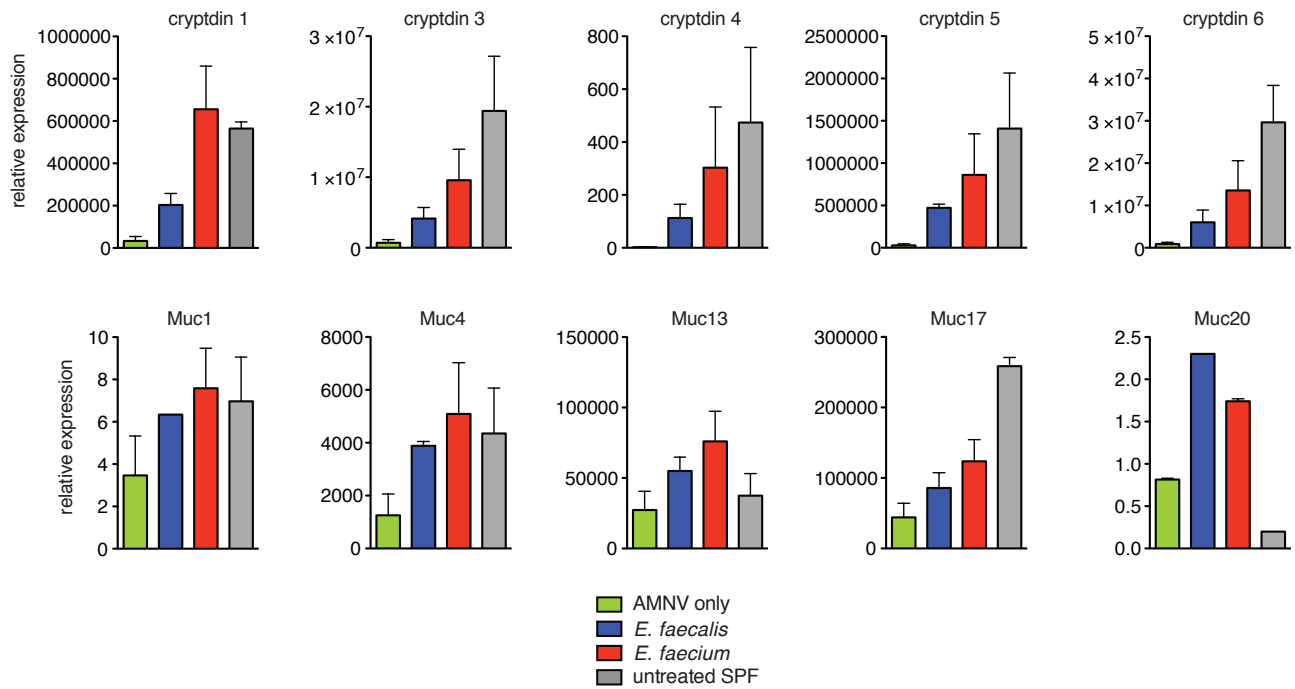


Figure S8. *E. faecium* elicits upregulation of antimicrobial peptides (AMPs). C57BL/6 mice were orally gavaged with AMNV daily for 7d prior to gavage with 10^8 CFU *E. faecalis* or *E. faecium*. 4d post-colonization, intestinal epithelial cells (IECs) were isolated and analyzed by RQ-PCR for expression of shown genes vs unmanipulated specific pathogen-free (SPF) mice. Representative data from 1 of 3 independent experiments, n=2-3 mice/group.

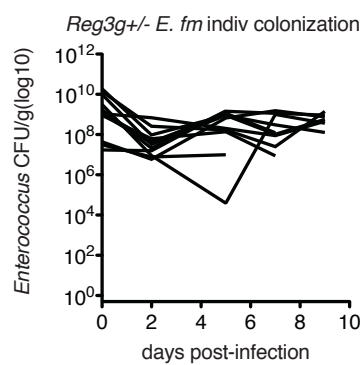
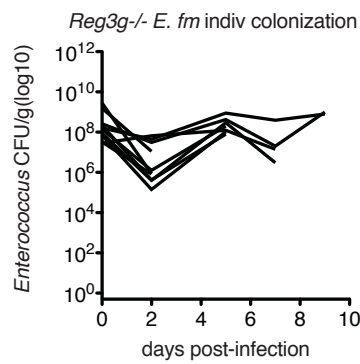
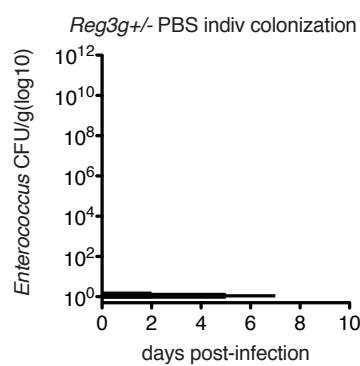
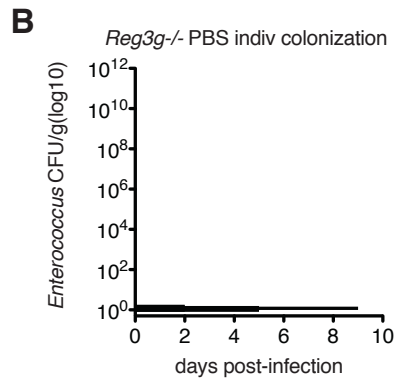
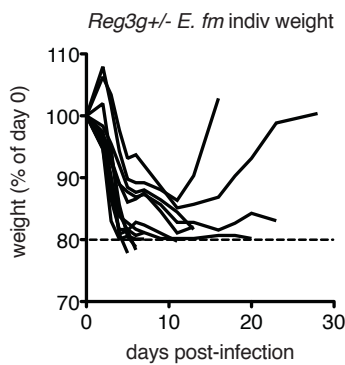
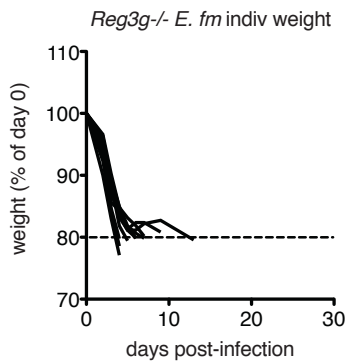
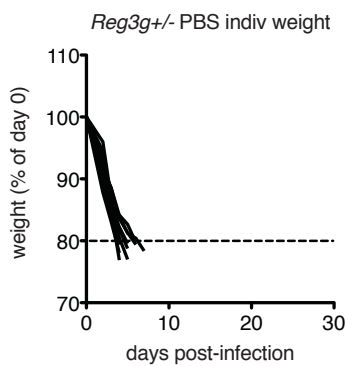
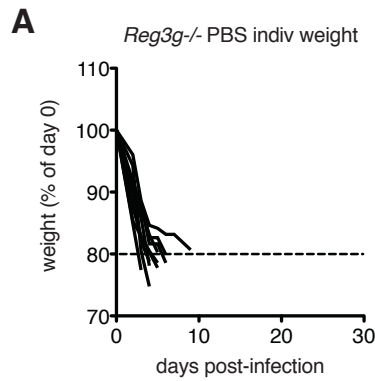


Figure S9. Individual weight loss and colonization of *Reg3g*^{-/-} mice. *Reg3g*^{-/-} mice or +/- littermate controls were given AMNV for 7d and colonized with 10^8 CFU *E. faecium* (*E. fm*) prior to oral infection with 10^6 CFU *S. Tm*. **(A)** Weight loss and **(B)** *E. fm* CFU in feces are shown for each mouse included in Fig. 4A-C. PBS-treated control mice did not exhibit any detectable *E. fm* in feces. Pooled data from 4 independent experiments, n=10-12 mice/group.

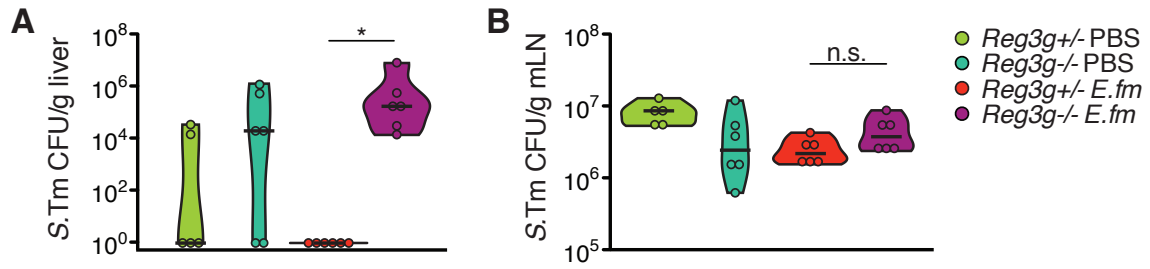


Figure S10. *Reg3g* is required for *E. faecium*-mediated decreases in *S. Typhimurium* invasion. *Reg3g*^{-/-} mice or +/- littermate controls were given AMNV for 7d and colonized with 10⁸ *E. faecium* (*E. fm*) prior to oral infection with 10⁶ *S. Tm*. **(A)** *S. Tm* bacterial burden 72h p.i. in the livers or **(B)** mesenteric lymph nodes (mLN) of *Reg3g*^{-/-} or +/- littermate controls. Pooled data from 2 independent experiments, n=5-6 mice/group. Bars=median, Wilcoxon (A) and Mann-Whitney (B) comparing *Reg3g*^{-/-} *E. fm* to *Reg3g*^{+/+} *E. fm*. *p≤0.05.

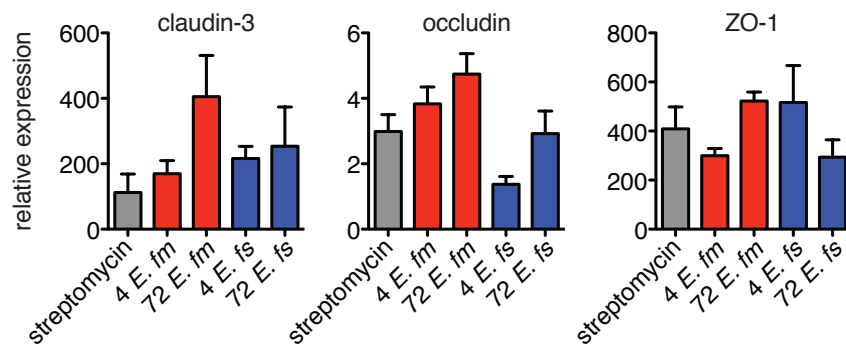


Figure S11. *E. faecium* does not significantly affect tight junction protein transcription.

C57BL/6 mice were orally gavaged with streptomycin and given sterile PBS or 10^8 CFU *E. faecalis* (*E. fs*) or *E. fm* 24h later. At 4 or 72h post-colonization IECs were isolated for analysis by RQ-PCR. Representative data from 1 of 3 independent experiments, n=2-3 mice/group. 1-way ANOVA with Dunnett's post-test comparing all to streptomycin-only control. All p-values were >0.05 and therefore not considered significant.

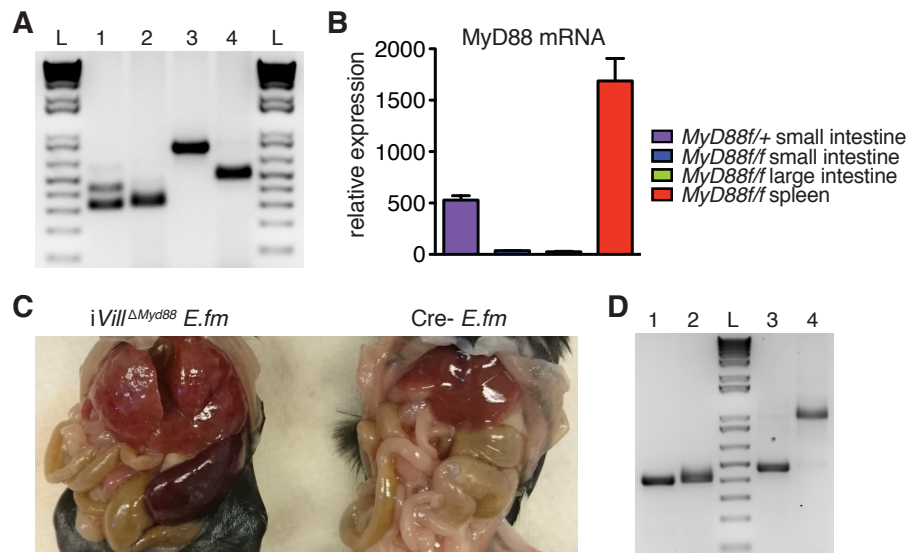
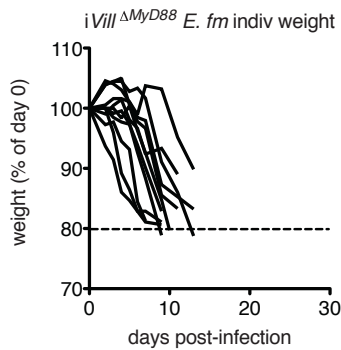
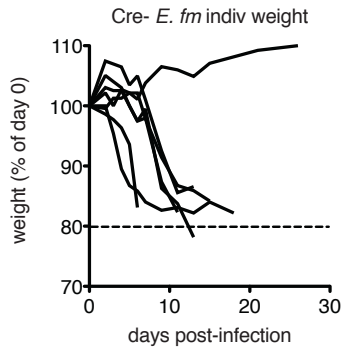
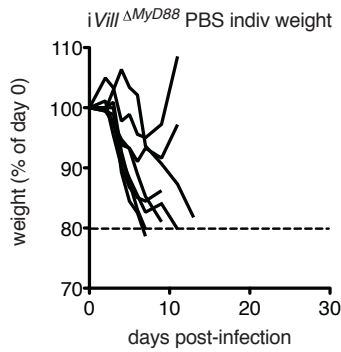
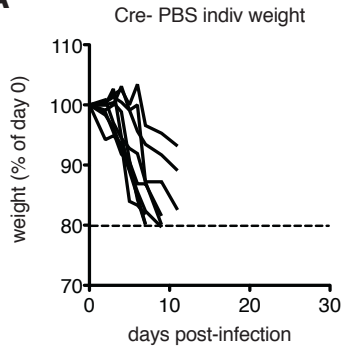
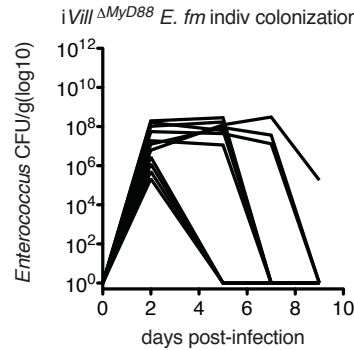
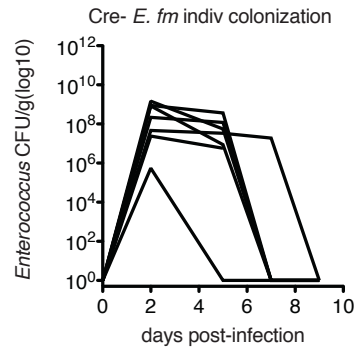
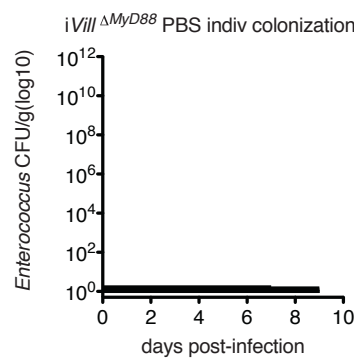
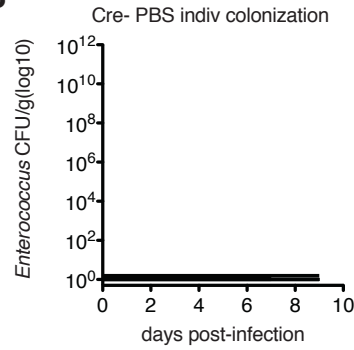


Figure S12. Tamoxifen induction of *MyD88* excision in *iVill*^{Δ*Myd88*} mice. (A and B) *iVill*^{Δ*Myd88*} mice were given a single i.p. injection of tamoxifen 5d prior to isolation of tissues for analysis by (A) PCR and (B) RQ-PCR. All mice were Cre⁺. For (A), lane L = ladder, 1&3 = *MyD88*^{f/+} small intestine, 2&4 = *MyD88*^{f/f} small intestine, using primers for the floxed allele (lanes 1&2) and excised or unexcised *MyD88* exons (lanes 3&4). The smaller-sized band in lane 4 indicates successful excision while the larger band in lane 3 indicates an intact allele. For (B) RNA was isolated, reverse transcribed and analyzed by RQ-PCR for tissue-specific loss of expression. **(C and D)** Co-housed *iVill*^{Δ*Myd88*} and Cre- (*MyD88*^{f/f}) littermate control were injected i.p. with tamoxifen daily for 2 consecutive days 7 days before gavage with streptomycin. 24h after streptomycin, mice were gavaged with 10⁸ CFU *E. fm* followed 4h later by infection with 10⁶ *S. Tm*. Mice were examined at 12d post-infection (C) and small intestine was collected to check *MyD88* excision by PCR (D). For (D), lane L = ladder, 1&3 = *iVill*^{Δ*Myd88*}, and 2&4 = Cre- (*MyD88*^{f/f}) littermate control, using primers for the floxed allele (lanes 1&2) and excised or unexcised *MyD88* exons (lanes 3&4).

A**B****Figure S13.****Individual weight loss and colonization of *iVill*^{ΔMyD88} mice.**

iVill^{ΔMyD88} or Cre⁻ (*MyD88*^{fl/fl}) littermate control mice were injected with tamoxifen 7 days prior to oral gavage with streptomycin. After 24h, mice were gavaged with PBS or 10⁸ CFU *E. fm* followed 4h later by oral infection with 10⁶ CFU *S. Tm*. (A) Weight loss and (B) *E. fm* CFU in feces are shown for each mouse included in Fig. 5A-C. PBS-treated control mice did not exhibit any detectable *E. fm* in feces. Pooled data from 3 independent experiments, n=7-11 mice/group.

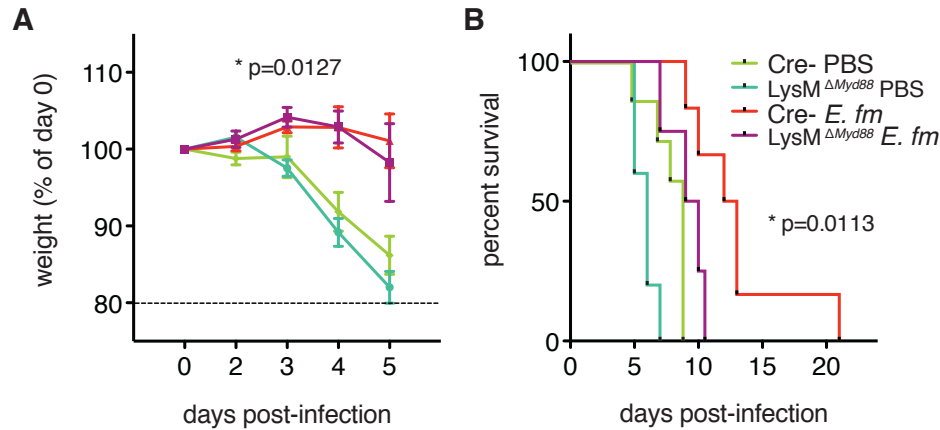


Figure S14. *E. faecium*-mediated protection is maintained in the absence of MyD88 signaling in myeloid cells. *LysMΔMyd88* or Cre⁻ (*MyD88*^{f/f}) littermate control mice were gavaged with streptomycin 24h prior to gavage with PBS or 10⁸ CFU *E. fm* followed 4h later by oral infection with 10⁶ CFU *S. Tm*. **(A)** Weight loss and **(B)** survival are shown. Pooled data from 2 independent experiments, n=4-7 mice/group. (A) mean±SEM, 2-way ANOVA, p-value shown comparing *E. fm*-treated *LysMΔMyd88* (purple) to PBS *LysMΔMyd88* controls (blue). (B) Log-rank analysis, p-value shown comparing *E. fm*-treated *LysMΔMyd88* (purple) to PBS *LysMΔMyd88* controls (blue). *p≤0.05 for all analyses.

Figure S15. Individual weight loss and colonization of *Nod2*^{-/-} mice. *Nod2*^{-/-} or +/- littermate control mice were gavaged with streptomycin 24h prior to gavage with 10⁸ CFU *E. fm* followed 4h later by oral infection with 10⁶ CFU *S. Tm*. **(A)** Weight loss and **(B)** *E. fm* CFU in feces are shown for each mouse included in Fig. 5E-G. PBS-treated control mice did not exhibit any detectable *E. fm* in feces. Pooled data from 4 independent experiments, n=9-12 mice/group.

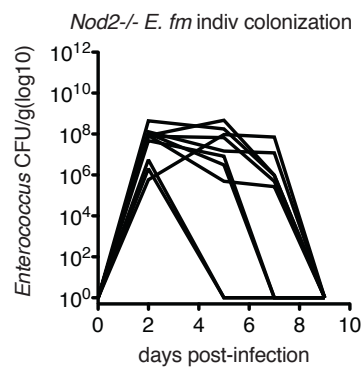
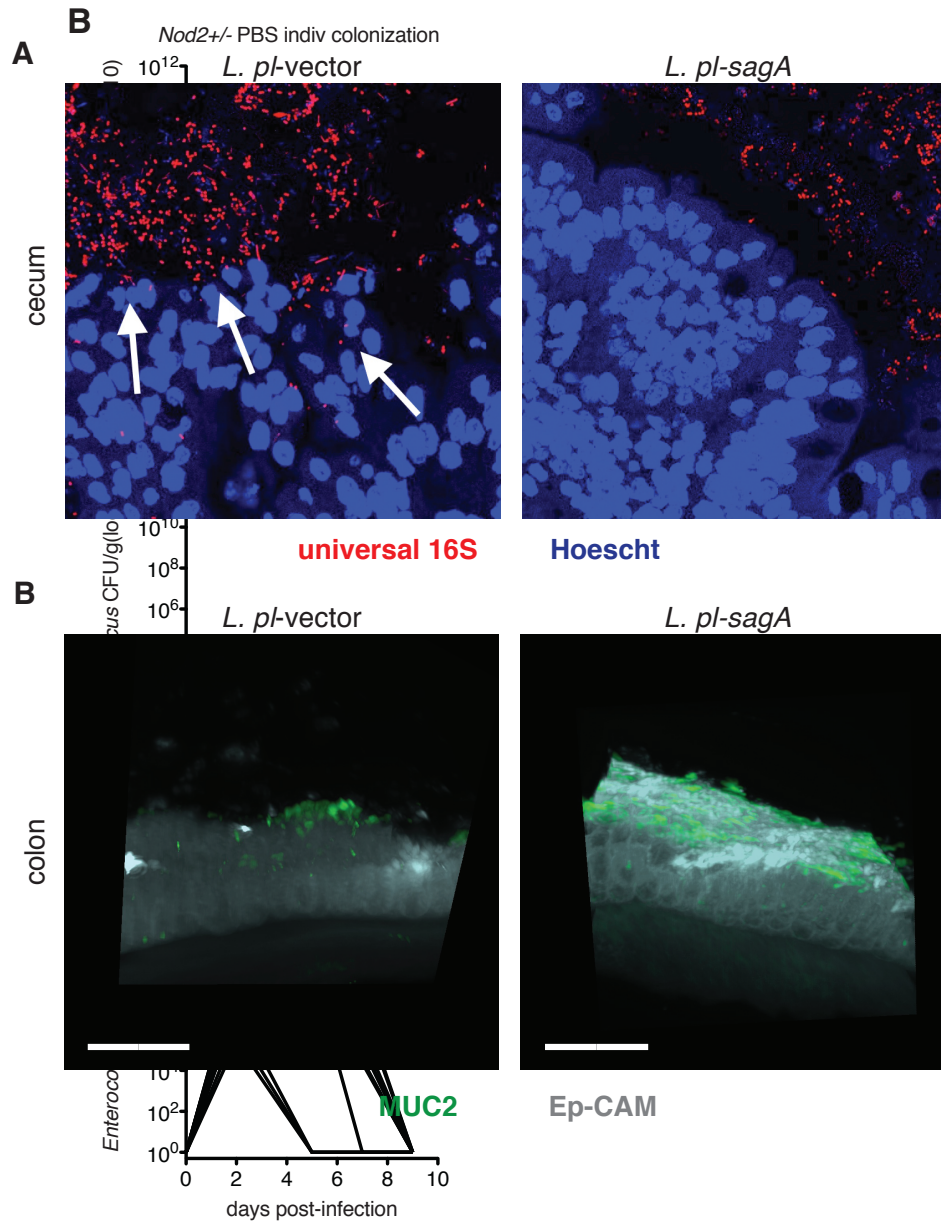
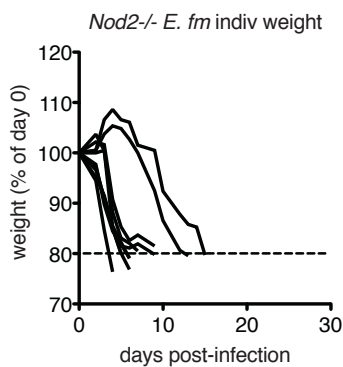
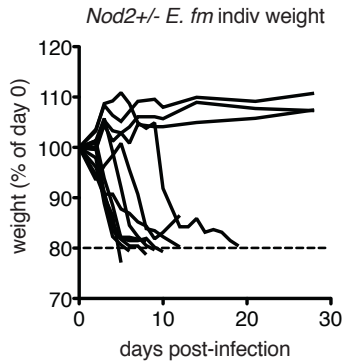
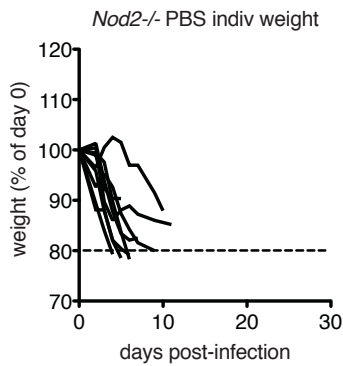
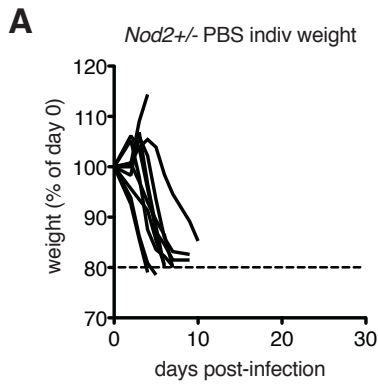


Figure S16. *L. plantarum-sagA* induces improved bacterial segregation and MUC2 distribution. C57BL/6 mice were orally gavaged with streptomycin and 24h later given 10^8 CFU *L. pl*-vector or *L. pl-sagA* 4h before oral

infection with 10^6 CFU *S. Tm*. Intestinal tissues were harvested at 48h p.i. **(A)** FISH staining for all bacteria (universal 16S probe) and epithelial nuclei (Hoechst). Representative images, 40X objective, from 1 of 3 independent experiments, n=6 mice/group. White arrows indicate bacteria in contact with or invading through the epithelium. **(B)** Captured images of tissue-cleared, MUC2- and Ep-CAM-stained colon from mice treated as in (A). Representative images from 2 independent experiments, n=4-5. Corresponding videos are Movies S7-8. Scale bar = 200 μ m.

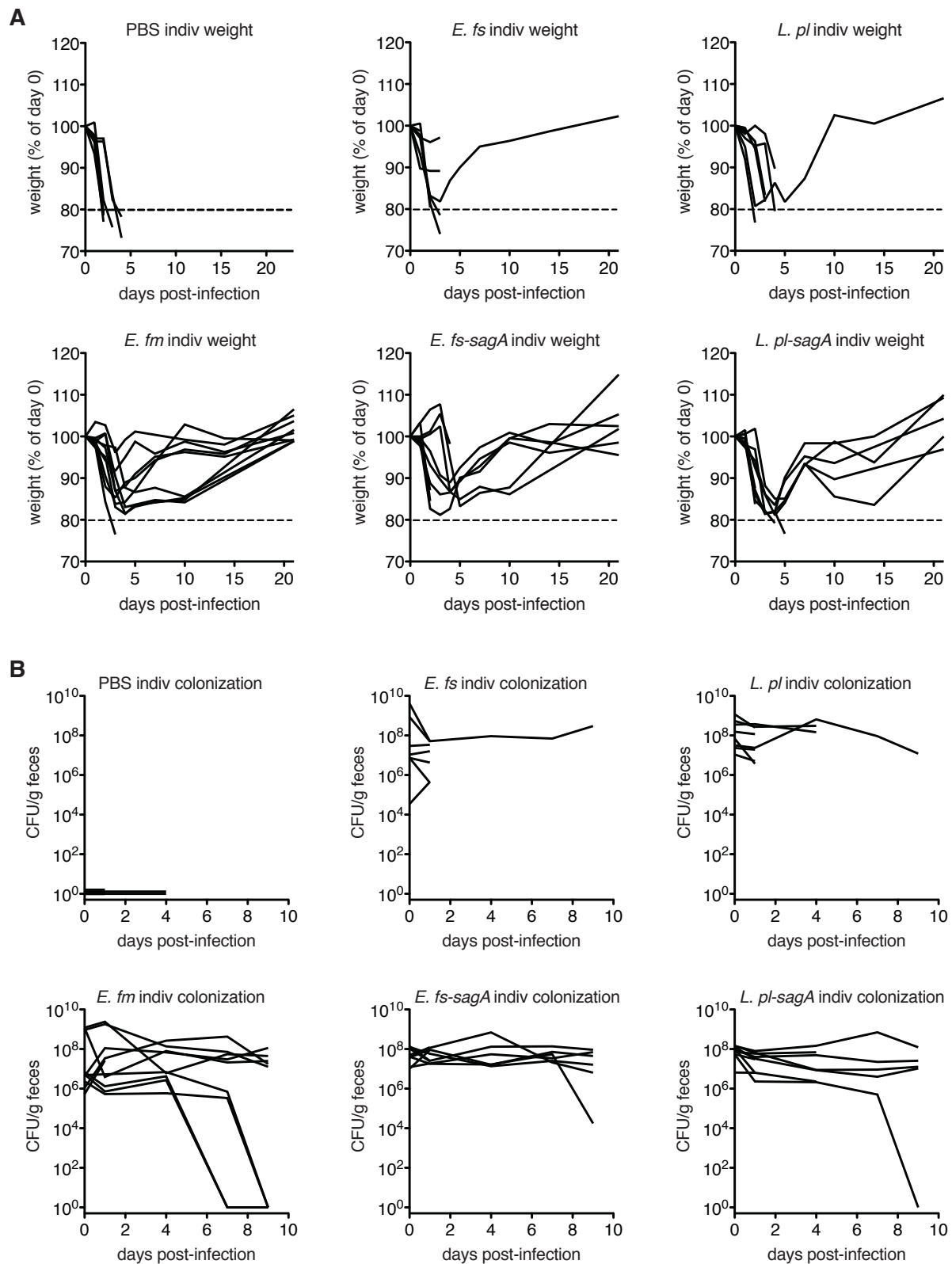


Figure S17. Individual weight loss and colonization of mice with *C. difficile* infection. C57BL/6 mice were given AMNV for 7d and colonized with 10^8 CFU of indicated bacteria 2d prior to oral infection with 10^6 *C. difficile*. **(A)** Weight loss and **(B)** CFU of indicated bacteria in feces are shown for each mouse included in Fig. 6F-H. Pooled data from 3 independent experiments, n=7-10 mice/group.

Movies S1-2. C57BL/6 mice were orally gavaged with streptomycin and 24h later given either sterile PBS or 10^8 CFU *E. fm* 4h before oral infection with 10^6 CFU *S. Tm*. Representative images from 2 experiments, n=7-8.

- **Movie S1.** Representative 3D imaging of tissue-cleared, MUC2- and Ep-CAM-stained colon 48h post-infection of PBS-treated mouse.
- **Movie S2.** Representative 3D imaging of tissue-cleared, MUC2- and Ep-CAM-stained colon 48h post-infection of *E. fm*-treated mouse.

Movies S3-6. *Reg3g*^{-/-} mice or *+/+* controls were given streptomycin 24h before gavage with either sterile PBS or 10^8 CFU *E. fm* followed by oral infection with 10^6 *S. Tm* 4h later. Representative images from 2 independent experiments, n=3-5.

- **Movie S3.** Representative 3D imaging of tissue-cleared, MUC2- and Ep-CAM-stained colon 48h post-infection of PBS-treated *Reg3g*^{+/+} mouse.
- **Movie S4.** Representative 3D imaging of tissue-cleared, MUC2- and Ep-CAM-stained colon 48h post-infection of PBS-treated *Reg3g*^{-/-} mouse.
- **Movie S5.** Representative 3D imaging of tissue-cleared, MUC2- and Ep-CAM-stained colon 48h post-infection of *E. fm*-treated *Reg3g*^{+/+} mouse.
- **Movie S6.** Representative 3D imaging of tissue-cleared, MUC2- and Ep-CAM-stained colon 48h post-infection of *E. fm*-treated *Reg3g*^{-/-} mouse.

Movies S7-8. C57BL/6 mice were orally gavaged with streptomycin and 24h later given 10^8 CFU *L. pl*-vector or *L. pl-sagA* 4h before oral infection with 10^6 CFU *S. Tm*. Representative images from 2 independent experiments, n=4-5.

- **Movie S7.** Representative 3D imaging of tissue-cleared, MUC2- and Ep-CAM-stained colon 48h post-infection of *L. pl*-vector-treated mouse.
- **Movie S8.** Representative 3D imaging of tissue-cleared, MUC2- and Ep-CAM-stained colon 48h post-infection of *L. pl-sagA*-treated mouse.